

Capto™ adhere

Multimodal chromatography resin

Instructions for Use

Capto™ adhere is a multimodal BioProcess™ resin for intermediate purification and polishing of monoclonal antibodies after capture on Protein A resin by packed bed chromatography. In combination with Protein A resin (i.e., MabSelect™ family), Capto adhere offers a robust chromatography platform for the development of monoclonal antibody manufacturing processes.

Capto adhere improves yield, productivity, and process economy with:

- high capacity in flow-through mode
- impurity reduction to formulation levels in post-Protein A purification:
 - leached Protein A
 - antibody dimers and aggregates
 - host cell proteins
 - nucleic acids
 - viruses
- wider operational window of pH and conductivity
- savings in time and operating costs with a two-step chromatographic process

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Important

Read these instructions carefully before using the product.

Safety

For use and handling of the product in a safe way, refer to the Safety Data Sheet.

1 BioProcess resins

BioProcess chromatography resins are developed and supported for production-scale chromatography. All BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist in process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

2 Resin properties

Capto adhere is a strong anion exchanger with multimodal functionality ([Fig. 1, on page 3](#)). The multimodal functionality gives a different selectivity compared to traditional anion exchangers.

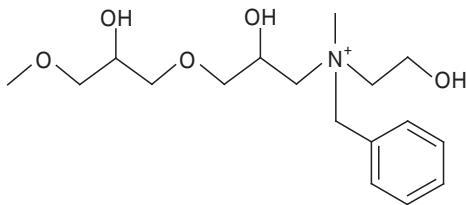


Fig 1. The Capto adhere ligand, N-Benzyl-N-methyl ethanol amine, exhibits many functionalities for interaction. The most pronounced are ionic interaction, hydrogen bonding, and hydrophobic interaction.

Capto adhere is designed for post-Protein A purification of monoclonal antibodies. Removal of leached Protein A, aggregates, host cell proteins, nucleic acids, and viruses from monoclonal antibodies is performed in flow-through mode where the antibodies pass directly through the column while the impurities are adsorbed.

The resin is based on a rigid high-flow agarose matrix that allows high flow velocities to be used ([Fig. 2, on page 4](#) and [Fig. 3, on page 5](#)). The highly cross-linked agarose base matrix gives the resin high chemical and physical stability. Characteristics such as capacity, elution behavior, and pressure-flow properties are unaffected by the solutions commonly used in process chromatography and cleaning procedures ([Table 1, on page 6](#)).

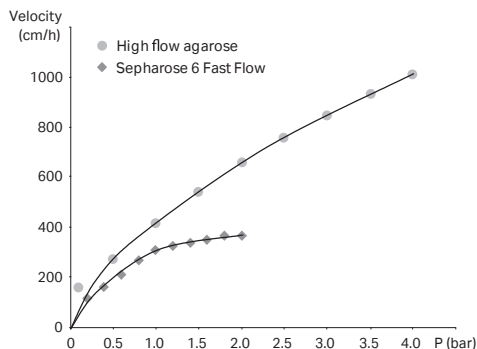


Fig 2. Pressure-flow characteristics of Capto adhere compared to Sepharose™ 6 Fast Flow. Running conditions: BPG™ 300 (30 cm i.d.) open bed at settled bed height of 20 cm, with water at 20°C.

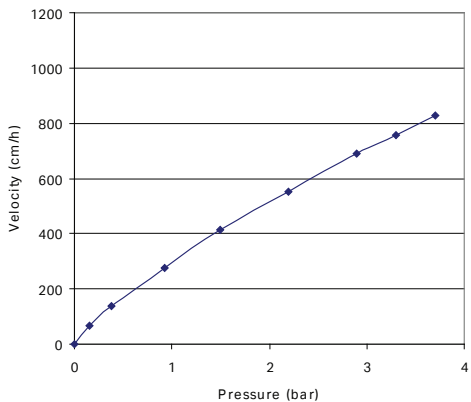


Fig 3. Pressure-flow characteristics of Capto adhere in a BPG 300, 20 cm packed bed with water at 20°C.

Table 1. Characteristics of Capto adhere

Matrix	Highly cross-linked agarose, spherical
Functional group	Multimodal strong anion exchanger
Ionic capacity	0.09 to 0.12 mmol Cl ⁻ /mL resin
Particle size, d_{50v} ¹	~ 75 µm
Pressure-flow characteristics ^{2,3}	≤ 600 cm/h at ≤ 0.3 MPa in a 1 m diameter column and 20 cm bed height (at 20°C using process buffers with the same viscosity as water)
pH stability	
Operational ⁴	3 to 12
CIP ⁵	2 to 14
pH ligand fully charged	Entire operational pH range
Working temperature ⁶	4°C to 30°C
Chemical stability ⁷	Stable in commonly used aqueous buffers, 1 M acetic acid, 1.0 M NaOH
Avoid	Oxidizing agents, anionic detergents
Autoclavability	17 min at 121°C in 0.05 M phosphate buffer, pH 7, 10 cycles

¹ Median particle size of the cumulative volume distribution.

² The capacity for selective reduction of some key impurities can decrease at high flow velocity.

³ The pressure-flow characteristics describe the relationship between pressure and flow under set circumstances. The pressure shall not be taken as the maximum pressure of the resin.

⁴ pH range where resin can be operated without significant change in function.

⁵ pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

⁶ Capto adhere can be used under cold-room conditions, but the capacity for some key impurities can decrease.

⁷ 1.0 M NaOH must only be used for cleaning purposes.

3 Method design and optimization

Introduction

Capto adhere is a multimodal ion exchanger and designed to be used as a second or third step in monoclonal antibody (mAb) purification processes, (i.e., after capture on a Protein A resin). Removal of leached Protein A, antibody dimers and aggregates (D/A), host cell proteins (HCP), viruses, and nucleic acids is preferably performed in flow-through mode where the antibodies pass directly through the column while the impurities are adsorbed.

General purification protocol

- Adjust pH and conductivity of the Protein A pool to loading conditions for flow-through mode.
- Equilibrate the column with loading buffer of the same pH and conductivity as the sample.
- Apply the sample onto the column. Collect the flow-through fraction.
- Wash out unbound material with loading buffer and collect together with the flow-through fraction.
- Regenerate column to elute bound material.
- Clean-In-Place.
- Re-equilibrate.

Sample preparation

Before sample loading, pH and conductivity of the sample must be adjusted to the target loading conditions. This is done either by buffer exchange or by direct adjustment of pH and conductivity. Buffers that are normally used for ion exchange chromatography can also be used for Capto adhere ([Table 2, on page 8](#)).

Table 2. Recommended buffers

pH interval	Buffer ^{1,2}	Concentration ³
4 to 5	Acetate	20 to 100 mM
4 to 6	Citrate	20 to 200 mM
5.5 to 6.5	Bis-TRIS	20 to 50 mM
6 to 7.5	Phosphate	50 to 200 mM
7.5 to 8.5	TRIS	20 to 50 mM
8.5 –	Glycin-NaOH	20 to 100 mM

¹ The choice of buffer systems and salts can influence both yield and impurity reduction.

² Buffers in the pH interval 5.5 to 8 will normally be most efficient for impurity reduction.

³ Conductivity can be adjusted by addition of salt or by varying the buffer concentration.

Buffer exchange

For preparation of well defined samples for optimization of loading conditions at laboratory scale, buffer exchange can be performed on HiPrep™ 26/10 Desalting¹. A typical chromatogram is shown in [Fig. 4, on page 9](#).

¹ Prepacked with Sephadex™ G-25 Fine to provide reliable and reproducible desalting and buffer exchange with sample sizes up to 15 mL per column.

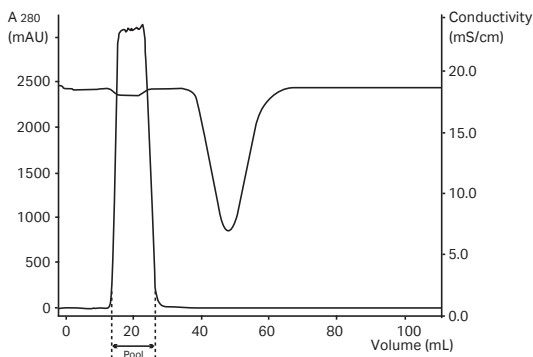


Fig 4. Buffer exchange on HiPrep 26/10 Desalting. Sample volume: 10 mL MabSelect SuRe™ eluate. Buffer exchange to 25 mM BIS-TRIS, 175 mM NaCl, pH 7.0. Flow rate: 15 mL/min. Pooled material: 13 mL.

Conductivity and pH adjustment

For larger volumes of feed, sample preparation is preferably performed by diafiltration or directly by adjustment of pH and conductivity².

²

Buffer exchange can result in reduction of HCP levels and improve column performance.

Initial screening of loading conditions

Product yield and loading conditions

Balancing product yield against product purity is the major consideration when optimizing a method. When running in flow-through mode, loading conditions will usually be a compromise between conditions favoring yield and conditions favoring impurity reduction. By adjusting pH and conductivity of the sample as well as the sample load, conditions can be obtained where most impurities are adsorbed while the monomeric antibodies pass through the column.

Optimization of loading conditions is preferably performed by using Design of Experiments (DoE). A common approach in DoE is to define a reference experiment (center point) and to perform representative experiments around that point. To be able to define the center point and the variable ranges some initial experiments are required.

Initial experiments

To find suitable DoE conditions, initial experiments can be performed in binding mode, using a pH gradient for elution (left part of [Fig. 5, on page 11](#)). The elution position, that is pH at peak maximum, defines the lower pH in the design. The upper pH in the design is normally about two pH units higher. Experiments can also be performed in flow-through mode, keeping the conductivity constant at a moderate level.

A comparison of chromatograms is shown in [Fig. 5, on page 11](#). At high pH (i.e., close to pI for the antibodies) the breakthrough during sample load is delayed, the breakthrough and wash curves are shallow, and significant amounts of mAb bind to the adsorbent. A decrease in pH (i.e., further away from pI) results in weaker electrostatic interaction between the antibodies and the adsorbent, steeper breakthrough and wash curves, and increased yield.

In the DoE, pH, conductivity, and load must be included. It is important to include conditions at the higher pH range resulting in lower yield and higher purity as well as conditions at the lower pH range resulting in higher yield and lower purity. An example is given below.

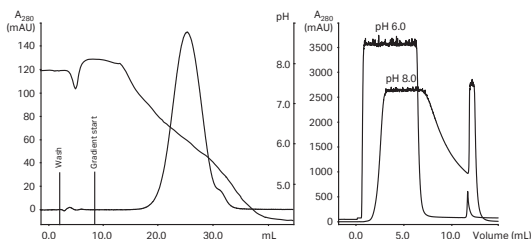


Fig 5. Sample: rProtein A elution pool, after buffer exchange on HiPrep 26/10 Desalting. Binding mode (left): Sample loading at pH 7.8. Load: 1 mg/mL resin. Elution performed in a pH gradient from 7.8 to 4.0. Flow-through mode (right): Comparison of chromatograms obtained at different pH levels. Load: 75 mg mAb/mL resin. Conditions: pH 8.0, 2 mS/cm and pH 6.0, 2 mS/cm.

DoE for optimization of loading conditions

DoE is recommended for optimization of loading conditions. By systematically varying important parameters (i.e., pH, conductivity, and sample load) response surfaces can be obtained for yield and for reduction of key impurities.

The table below describes a procedure for setting up a full factorial DoE with three parameters:

Step	Purpose	Description
1	Work prior to actual setup of the design.	Perform initial loading experiments at varying pH, as described above. Choose parameters to include and define parameter ranges and responses.
2	Choose design for screening or optimization.	Full factorial design is commonly used in both screening and optimization. A full factorial DoE in 3 parameters will give $2^3 = 8$ experiments + center points.
3	Choose center points for the design.	Center points are important in DoE because they give an indication if there is curvature in the data. Three replicated center points are recommended. A full factorial design in three parameters with three center points gives a total of 11 experiments.
4	Systematic variation of the parameters.	Use limiting values, high and low, of each parameter. To be able to separate effects, combine the high and low values in a way that makes the parameters independent of each other. For more information, see www.sartorius.com .

Note: A module for planning and evaluation of DoE is included in UNICORN™. The module works only with ÄKTA avant™ and UNICORN 6 and higher.

Example of high-throughput screening and optimization

As an alternative to the use of small columns, high-throughput process development (HTPD) tools like PreDicator™ 96-well plates can be used for process optimization. An example of this is described in application note *High-throughput screening and optimization of a multimodal polishing step in a monoclonal antibody purification process (CY13312)*.

Description

The application note describes the development and optimization of a polishing step for the purification of a monoclonal antibody on Capto adhere. In the initial part of this study, PreDicator 96-well plates prefilled with Capto adhere were used to screen a large experimental space quickly. Promising results from the plate study were further optimized with HiScreen™ columns and the DoE approach to establish the final process conditions.

Results

Application of the optimized protocol led to a reduction in aggregate levels from 12.6% to < 0.5% in a single step with a monomer yield of 87%. HCP and ligand leakage were reduced to negligible amounts. In total, 192 conditions (flow-through and selective elution experiments) were screened in approximately 4 h and analyzed in 48 h.

Example of DoE performed in columns

Sample

Start material: Monoclonal IgG1 expressed in CHO cell culture supernatant, initially purified on rProtein A resin.

Sample characteristics: pI $\approx$ 9, leached Protein A 36 ppm, D/A 3.3%³ and HCP 210 ppm.

Experimental setup

The experimental setup was a full factorial design^{4,5}. The three factors studied were load, pH, and conductivity, with additional points to resolve curvature effects ([Table 3, on page 15](#)). In all, the number of experiments included in the model was 14, and the measured responses were yield (%) and Protein A (ppm), dimer/aggregate (%) and HCP (ppm) in the flow-through pool. For each response a separate model was calculated. The models were fitted to MLR (multiple linear regression) and are well explained and show good stability to cross-validation.

³ As determined by analytical size exclusion chromatography on Superdex™ 200/300 GL.

⁴ Experiments were designed and evaluated using MODDE 7.0 software (Sartorius).

⁵ www.sartorius.com

Table 3. Design setup¹

Load (mg mAb/mL)	pH	Conductivity (mS/cm)
75	6	2
300	6	2
75	8	2
300	8	2
75	6	15
300	6	15
75	8	15
300	8	15
187.5	7	8.5
187.5	7	8.5
75	7	15
300	7	15
187.5	7	2
187.5	7	15

¹ The design setup includes 2 center points (bold) and 4 additional points at pH 7 to resolve curvature effects.

Parameters affecting the yield

The parameters that affect the yield are shown in the coefficient plot ([Fig. 6, on page 16](#)). The plot shows that high sample load, low pH, and high conductivity result in high yield. The interaction effects (load × pH and load × conductivity) are also significant for the yield response. The response surfaces also show that higher loads will give a larger pH window with yield > 90%.

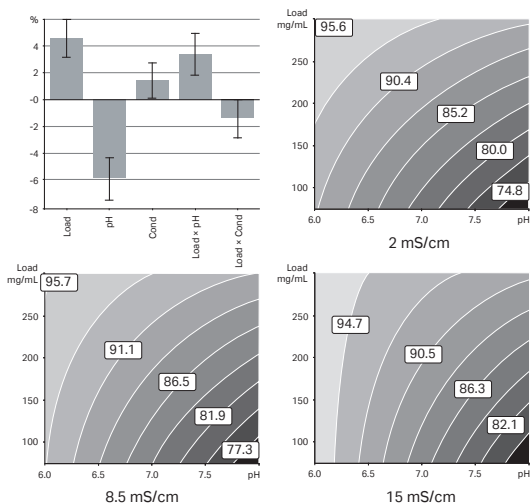


Fig 6. Coefficient plot and response surfaces for the yield model. Load versus pH at different conductivities.

The coefficient plot describes the impact on the response from the investigated parameters. In the case above, the following can be seen:

- Load is positively correlated to the response – meaning that a higher load will give a higher value on the response.
- pH is negatively correlated to the response – meaning that a lower pH will give a higher value on the response.
- Conductivity is positively correlated to the response, but to a lesser degree – meaning that a higher conductivity will also give a higher response value, but not to the same extent as the effect of load.

One way to further improve yield is to perform selective elution of monomers. For further information, see Application note *High-throughput screening and optimization of a multimodal polishing step in a monoclonal antibody purification process (CY13312)*.

The interaction effects that are present in the coefficient plot (load $\times$ pH and load $\times$ conductivity) mean that if, for example, the pH is changed, the response will not only be changed with the effect of the pH but also with the effect of the load at that specific pH. The same goes for the load $\times$ conductivity interaction.

Parameters affecting Protein A clearance

The coefficient plot shows that high pH will give good Protein A clearance. The conductivity by itself did not significantly affect the response, but there is a significant interaction effect for pH $\times$ conductivity ([Fig. 7, on page 18](#)). If this term is high, Protein A clearance will be low. Load was not a significant factor for this response.

The response surfaces show that high pH and low conductivity will give high Protein A clearance.

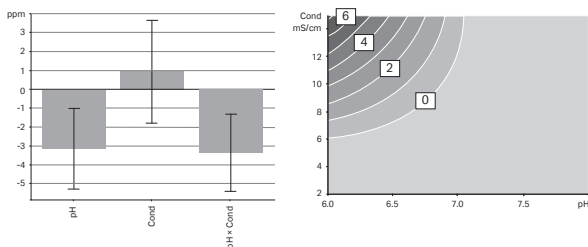


Fig 7. Coefficient plot and response surfaces for the Protein A model. Conductivity versus pH. Protein A concentration expressed in ppm.

Parameters affecting dimer/aggregate clearance

The coefficient plot ([Fig. 8, on page 19](#)) shows that pH is the most important parameter and that high pH will give low D/A concentration in the flow-through pool. The load parameter is also significant, but very small. A low load gives high D/A clearance. There is also a significant curvature effect assigned to pH. If pH is too high or too low, the D/A response will increase. The conductivity did not significantly affect D/A clearance. The response curve shows that the load only has a small effect on D/A clearance, so only pH has to be considered.

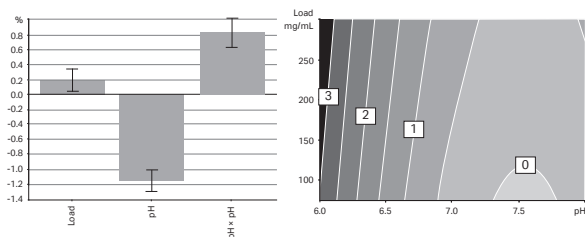


Fig 8. Coefficient plot and response surface for the D/A model. Load versus pH. D/A concentration expressed in percent.

Parameters affecting HCP clearance

The coefficient plot ([Fig. 9, on page 20](#)) and response curves show that low sample load, low conductivity, and high pH will give low HCP values.

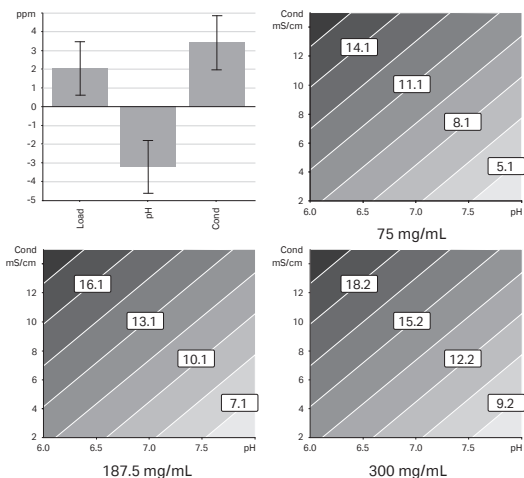


Fig 9. Coefficient plot and response surfaces for the HCP model. Conductivity versus pH. HCP concentration expressed in ppm.

Conclusions

The response surfaces above show the influence of sample load, pH, and conductivity on four different responses (yield of monomeric mAb and clearance of Protein A, dimer/aggregates and HCP, respectively), and how to reach the target values for each of them.

Even though the optimal conditions for each response are not the same, there is quite a large area where acceptable values can be obtained for all four responses. Suggested loading conditions for this mAb could be a sample load of 200 mg/mL, pH 7, and conductivity 8.5 mS/cm.

General trends

Each monoclonal antibody is unique, and the level of impurities varies between different cell lines and differences in previous purification steps. This implies that it can be difficult to predict optimal loading conditions. However, based on DoE performed with several different antibodies, some general trends have been identified ([Fig. 10, on page 22](#)).

- Best yield: High load, low pH, and high conductivity
- Best D/A clearance: High pH, low load, and low conductivity. D/A clearance is often less affected by conductivity than Protein A and HCP clearance.
- Best Protein A and HCP clearance: High pH and low conductivity

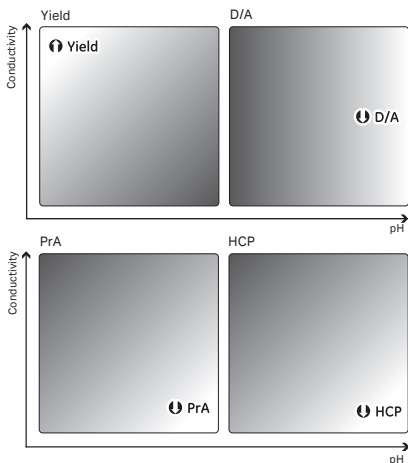


Fig 10. General trends with respect to loading conditions for yield, D/A, Protein A, and HCP clearance.

Loading conditions will therefore be a compromise between conditions favoring yield and conditions favoring impurity reduction. Optimal loading conditions will be a balance between load, pH, and conductivity. Consequently, for optimization of the loading step, vary all three parameters in the same experimental series.

In [Table 4, on page 23](#), optimal loading conditions for five different antibodies are shown. As can be seen, pH is normally below the isoelectric point, while optimal conductivity is harder to predict.

Table 4. Optimal loading conditions for different mAbs

mAb	pI	pH	Conductivity (mS/cm)
mAb 1	≈ 9	7.0	8
mAb 2	8.3 to 8.9	5.5	3
mAb 3	7.5 to 8.4	6.0	2
mAb 4	7.7 to 8.0	7.0	20
mAb 5	6.5 to 9.0	7.7	20

Bed height, flow velocity, and residence time

Typical bed height range at production scale is 10 to 20 cm. Recommended flow velocity is between 150 and 600 cm/h⁶, and the residence time⁷ 2 minutes⁸, or more. Longer residence times can result in more efficient impurity reduction.

As the loading capacity in flow-through mode is relatively high (100 to 300 mg mAb/mL resin), lower bed heights are preferably used at laboratory scale. However, the flow velocity must be adapted to a suitable residence time. Prepacked 1 mL and 5 mL HiTrap™ Capto adhere columns are available for screening of loading and elution conditions, and for method scouting.

⁶ 1 m diameter, 20 cm bed height at 20°C using buffer with the same viscosity as water.

⁷ Residence time, $\tau = L/u$, where L is the bed height in cm and u is the linear velocity (cm/h).

⁸ Corresponding to 600 cm/h at 20 cm bed height.

Wash out unbound material

After sample load, continue to wash out unbound mAb with loading buffer until the UV curve starts to level off. The wash fractions can normally be pooled together with the flow-through fractions. How much of the wash fractions that is collected is a balance between yield and dilution of the pooled material.

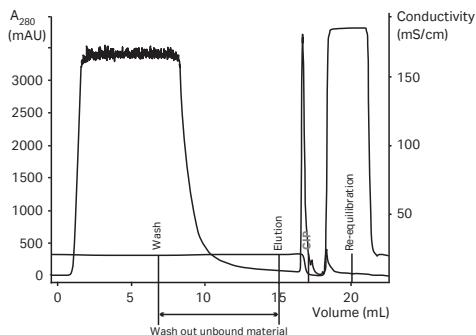


Fig 11. Example of wash after sample application.

Regeneration, Cleaning-In-Place, and re-equilibration

After wash-out of unbound material, perform regeneration at low pH as described in [Chapter 6 Maintenance, on page 44](#).

Cleaning-In-Place (CIP) is recommended before first-time use – especially after packing the column – or after long-term storage. CIP is also recommended after each cycle. A standard protocol for CIP is to wash with at least 3 column volumes of 1.0 M NaOH, using a contact time of 15 minutes. For further details, see [Chapter 6 Maintenance, on page 44](#).

At the end of each purification cycle, and after regeneration and CIP, re-equilibrate the column with 5 bed volumes of loading buffer, or until the column effluent shows stable conductivity and pH values.

4 Column packing

Recommended columns

Table 5. Recommended columns for Capto adhere

Column	Inner diameter (mm)	Bed volume ¹	Bed height (cm)
Laboratory scale			
Tricorn™ 5/100	5	2 mL	10
Tricorn 10/100	10	8 mL	10
HiScale™ 10/40	10	8 to 20 mL ²	max 25 ²
HiScale 16/20	16	20 to 40 mL	max 20
HiScale 16/40	16	20 to 70 mL	max 35
HiScale 26/20	26	53 to 106 mL	max 20
HiScale 26/40	26	53 to 186 mL	max 35
HiScale 50/20	50	196 to 393 mL	max 20
HiScale 50/40	50	196 to 687 mL	max 35
Production scale			
AxiChrom™	50 to 200	0.2 to 12.5 L	max 40
AxiChrom	300 to 1000	7 to 314 L	max 50
BPG ³	100 to 300	1 to 28 L	max 40
Chromaflo™ standard ⁴	400 to 800	12 to 151 L	max 30

¹ Bed volume range calculated from 10 cm bed height to maximum bed height.

² Packing methods for bed heights up to 25 cm are provided.

³ The pressure rating of BPG 450 is too low to use with Capto resins.

⁴ Larger pack stations might be required at larger diameters.

All production-scale columns can be supplied as variable bed height columns. Do not choose large diameter columns if the bed height is low.

For more details about packing HiScale columns, see instructions *HiScale columns (10, 16, 26, 50) and accessories* (28967470).

Packing Tricorn 5/100 and 10/100 columns

Materials needed

Capto adhere
Glass filter funnel
Plastic spoon
Filter flask
Measuring cylinder
10 mM NaCl in purified water

Calculating amount of resin

The amount of Capto adhere resin needed can be calculated by: column cross-sectional area (cm^2) $\times$ bed height (cm) $\times$ compression factor (settled resin bed height/packed resin bed height).

Washing the resin

Equilibrate all materials to room temperature. Mount the glass filter funnel onto the filter flask. Pour the resin into the funnel and wash 5 times with 1–2 mL 10 mM NaCl per mL resin.

Preparing the packing slurry

The slurry concentration must be 40% to 60% in 10 mM NaCl, measured in a measuring cylinder after settling overnight.

Equipment needed

An ÄKTA™ design system or a stand-alone pump that can deliver 40 mL/min is used. The pump filter unit and the flow restrictor must be removed due to the high flow velocity used during column packing in order to decrease system backpressure.

For packing Tricorn 5/100 column: Tricorn 5/100 column, Tricorn 5/100 glass tube (used as a packing reservoir), packing connector 5-5, and bottom unit.

For packing Tricorn 10/100 column: Tricorn 10/100 column, Tricorn 10/100 packing equipment, which includes the 10 mm packing connector, Tricorn 10/100 glass tube (used as a packing reservoir), and bottom unit with filter holder, cap, and stop plug.

When working with large sample volumes or when repeatedly loading sample, the Tricorn coarse filter kit is recommended to reduce the risk of clogging. Use Tricorn 5 Coarse filter kit (11001253) or Tricorn 10 Coarse filter kit 10 (11001254).

Packing procedure

To pack the column, use 10 mM NaCl and proceed as follows:

Step	Action
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- | | |
|---|--|
| 1 | Assemble the column according to the column instructions (<i>Tricorn Empty High Performance Columns (28409488)</i>). |
|---|--|

Step	Action
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- | | |
|---|---|
| 2 | Pour the chromatography resin into the top of the packing tube filling both column tube and packing reservoir. Remove all air bubbles. |
| 3 | Attach a bottom unit or an adapter unit to the top of the packing tube. Place a beaker beneath the column and connect a pump to the top of the column, remove the stop plug from the bottom of the column tube. |
| 4 | Settle the resin at 0.25 mL/min (Tricorn 5/100) or 1 mL/min (Tricorn 10/100) for 10 minutes or until the bed has settled. |
| 5 | After the bed has settled, raise to packing flow – 10 mL/min (Tricorn 5/100) or 40 mL/min (Tricorn 10/100) for 2 minutes. |
| 6 | When the resin is packed, switch off the pump, attach the stop plug into the bottom of the column tube, disconnect the column, and remove the packing reservoir and packing connector. Let the liquid in the packing reservoir pour down the column and into the beaker. If necessary, carefully remove excess resin by re-suspending the top of the packed bed and remove with a Pasteur pipette or spatula. |
| 7 | Top off the column with the same fluid as used for packing the column. |

Step	Action
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- | | |
|---|---|
| 8 | Place a pre-wet filter on top of the fluid in the column. |
|---|---|

Note:

The top coarse filter is inserted by another procedure. See separate instruction included in the coarse filter kit.

- | | |
|---|---|
| 9 | Prepare the adapter unit by screwing the guiding ring inside the adapter unit out to its outer rim and then back 1.5 turns. |
|---|---|

- | | |
|----|---|
| 10 | Wet the O-ring on the adapter unit by dipping it into water, packing buffer or 20% ethanol. |
|----|---|

- | | |
|----|--|
| 11 | Screw the adapter unit onto the column tube, making sure that the inner part of the guiding ring fits into the slot on the column tube threads. Make sure that there are no air bubbles. |
|----|--|

Note:

Although it is possible to fit the adapter unit on the column tube without keying the inner locking device into the slot on the column tube, the adapter lock will not function. The consequence of this is that the adapter is not locked in position and accidental turning of the adapter is possible.

- | | |
|----|--|
| 12 | Screw the adapter down to approximately 1 mm above the surface. |
| 13 | Connect the pump to the adapter unit. Remove the stop plug from the bottom of the column tube. |

Step	Action
14	Pack the column at 10 mL/min (Tricorn 5/100) or 40 mL/min (Tricorn 10/100) for an additional 15 minutes.
15	Before switching off the pump, mark the position of the resin surface on the column with a pen.
16	Switch off the pump, attach the stop plug into the bottom of the column tube, and disconnect the column from the pump. Screw the adapter unit down to the marking.
17	Press the adapter lock down into the locked position.
18	Screw a stop plug into the adapter unit.

The column is now ready to be tested.

Note: *It is recommended to perform CIP after packing, as column packing involves open handling of the resin.*

Testing the packed column

See [Chapter 5 Evaluation of column packing, on page 41](#).

Packing HiScale columns

Materials needed

Capto adhere

HiScale column

HiScale packing tube (depending on bed height)

Plastic spoon or spatula

Glass filter G3

Vacuum suction equipment
Filter flask
Measuring cylinder
20% ethanol with 0.4 M NaCl

Equipment

Depending on the flow rate required, an ÄKTA system or a stand-alone pump such as Pump P-900, can be used for packing. Equilibrate all materials to room temperature.

Definitions

The height of a bed that has settled by gravity differs from the height of a bed that has settled at a given flow velocity (consolidated). Therefore, the compression factor (CF) must be separated from the packing factor (PF). The parameters for calculating the bed height are described below:

L_{settled}	Settled bed height (cm) Bed height measured after settling by gravity
L_{cons}	Consolidated bed height (cm) Bed height measured after settling at a given flow velocity
L_{packed}	Packed bed height (cm)
CF	Compression factor, $CF = L_{\text{settled}} / L_{\text{packed}}$
PF	Packing factor, $PF = L_{\text{cons}} / L_{\text{packed}}$
A_C	Cross-sectional area of the column (cm^2)
V_C	Column volume, $V_C = L_{\text{packed}} \times A_C$ (mL)
C_{slurry}	Slurry concentration (%)

Preparation of the slurry

To measure the slurry concentration, let the resin settle in 20% ethanol at least overnight in a measuring cylinder or use the Slurry Concentration Kit (29096100).

Washing the resin

Mount a glass filter funnel onto a filter flask. Suspend the resin by shaking it, pour it into the funnel, and wash it as follows:

- 5 times with 1–2 mL 20% ethanol with 0.4 M NaCl/mL resin.
- Gently stir with a spatula between additions.
- Transfer the washed resin from the funnel to a beaker and add 20% ethanol with 0.4 M NaCl to obtain a 50% slurry concentration. For HiScale 10/40, prepare a 60% slurry concentration.

Main packing parameters

Table 6. Main packing parameters for HiScale 10/40

Parameter	HiScale 10/40		
Bed height (cm)	10	20	25
Slurry/packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Settling velocity (cm/h)	76	76	76
Settling flow rate (mL/min)	1	1	1
Packing factor ¹ (PF)	1.03	1.03	1.03
Packing flow velocity (cm/h)	1910	1910	1910
Packing flow rate ² (mL/min)	25	25	25
Conditioning flow velocity with upflow (cm/h)	1528	1528	1528
Conditioning flow rate with upflow ³ (mL/min)	20	20	20

¹ The packing factor stated refers to mechanical compression of the bed. See introductory information about HiScale 10 columns and Capto adhere in [Packing procedure on page 36](#).

² For 5 column volumes.

³ For 10 column volumes.

Table 7. Main packing parameters for HiScale 16/20 and HiScale 16/40

Parameter	HiScale 16/20	HiScale 16/40	
Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.10	1.10	1.02
Packing flow velocity (cm/h)	750	750	750
Packing flow rate (mL/min)	25	25	25
Conditioning flow velocity (cm/h)	750	750	420
Conditioning flow rate (mL/min)	25	25	14

Table 8. Main packing parameters for HiScale 26/20 and HiScale 26/40

Parameter	HiScale 26/20	HiScale 26/40	
Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.15	1.10	1.03
Packing flow velocity (cm/h)	750	750	750
Packing flow rate (mL/min)	66	66	66
Conditioning flow velocity (cm/h)	750	750	420
Conditioning flow rate (mL/min)	66	66	37

Table 9. Main packing parameters for HiScale 50/20 and HiScale 50/40

Parameter	HiScale 50/20	HiScale 50/40	
Bed height (cm)	10	20	35
Slurry/packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.15	1.15	1.03
Packing flow velocity (cm/h)	750	750	750
Packing flow rate (mL/min)	250	250	250
Conditioning flow velocity (cm/h)	750	750	420
Conditioning flow rate (mL/min)	66	250	140

Packing procedure

For HiScale 10/40 columns, compression to target bed height is performed by using either a combination of flow compression and mechanical compression or flow compression only. However, when packing HiScale 10 columns with Capto adhere resin, compression to target bed height is performed in multiple steps. Compression starts with a settling step, followed by a flow compression step and a mechanical compression step, and ends with a flow conditioning step with an upflow (steps 22 to 27 in the procedure below).

Step	Action
------	--------

- | | |
|---|---|
| 1 | Assemble the column according to the column instructions <i>HiScale columns (10, 16, 26, 50) and accessories (28967470)</i> . |
| 2 | Attach the column tube to a stand. |
| 3 | Connect the bottom adapter unit to the pump or a syringe and prime the bottom net with a slow flow of packing solution. This is easiest done if the nets are dry but if air is trapped under the net it can be removed by a light suction with a syringe. |
| 4 | Attach the bottom adapter unit in the bottom of the column tube and tighten the O-ring firmly. |
| 5 | Fill the column with approximately 1 cm packing liquid using the pump/syringe. Disconnect the pump/syringe and attach a stop plug in the outlet. |
| 6 | Install the packing tube on top of the column tube if needed to achieve the required bed height. |
| 7 | Connect the top adapter to the pump and prime it with a slow downward flow. The net needs to be facing up as this is done. Any air trapped under the net can be removed by light suction with a syringe. |
| 8 | Fill the column with slurry suspended in packing solution. If needed, top up the slurry with extra packing solution so the top adapter dips into the slurry to avoid air under the net. |

Step	Action
------	--------

- | | |
|----|--|
| 9 | Install the top adapter unit on top of the packing tube. Tighten the O-ring firmly and remove the bottom stop plug. |
| 10 | Start a downward flow with packing velocity according to Table 6, 7, 8, or 9 . |
| 11 | Let the flow run until the bed has consolidated.
For HiScale 10/40, proceed to step 22. |
| 12 | Use the graduation marks on the column to measure the bed height. There might be a buildup of resin at the column wall after the bed is consolidated. To easier see where the top of the bed is, a light source can be used. |
| 13 | Calculate the final bed height by dividing the consolidated bed height with the recommended packing factor. $L_{\text{packed}} = L_{\text{cons}} / \text{PF}$. See Table 6, 7, 8, or 9 . |
| 14 | Turn off the flow and place a stop plug in the bottom. |
| 15 | Dismount the top adapter from the packing tube. |
| 16 | Over a beaker or a sink, detach the packing tube from the column. |
| 17 | Remount the top adapter in the column tube. Make sure that no air is trapped under the net and lower the adapter down to 1 to 2 cm above the bed, making sure that the surface is not disturbed. |

Step	Action
------	--------

18	Tighten the O-ring on the adapter. Remove the bottom stop plug and carefully start turning the end cap down. While spilling out liquid through the bottom, proceed turning until the calculated final bed height is reached.
----	--

19	Make sure that the pressure peaks that occur when turning the end knob down do not exceed the pressure specifications of the resin.
----	---

20	Start a downward flow to flow condition the bed. The flow rate is shown in Table 6, 7, 8, or 9 .
----	--

21	Let the flow run for about 10 column volumes.
----	---

Result:

The column is ready to be tested.

22	HiScale 10/40: Increase the flow to compress the bed. The flow is shown in Table 6, on page 34 . Continue this step for 5 column volumes (CV). At the end of this step, mark the bed height while keeping up the flow.
----	---

23	HiScale 10/40: Turn off the flow and untighten the top adapter slightly (release the pressure on the O-ring) which facilitates adjustment of the adapter without "twisting" the O-ring. This procedure should be performed with the bottom tubing open which makes it possible to monitor the pressure. The pressure that is generated from turning the adapter down must not exceed the pressure during the conditioning step. Continue to turn the adapter down until reaching the bed height mark.
----	--

Step	Action
------	--------

- | | |
|----|---|
| 24 | HiScale 10/40: Calculate the final bed height by dividing the consolidated bed height with the recommended packing factor, $L_{\text{packed}} = L_{\text{cons}}/\text{PF}$, see Table 6, on page 34 . |
| 25 | HiScale 10/40: Mechanically compress the bed to the final bed height according to the packing factor in the table. |
| 26 | HiScale 10/40: Before starting the conditioning flow, the O-ring should be firmly tightened. |
| 27 | HiScale 10/40: Perform the conditioning step for 10 CV with an upflow according to Table 6, on page 34 . |

Note:

For systems where an upflow cannot be selected, switch the column connections to the valve. Restore the original connections after the conditioning step and before testing the column.

Result:

The HiScale 10/40 column is ready to be tested.

Note: *It is recommended to perform CIP after packing, as column packing involves open handling of the resin.*

Testing the packed column

See [Chapter 5 Evaluation of column packing, on page 41](#).

5 Evaluation of column packing

Intervals

The packing quality needs to be checked by column efficiency testing. Perform column efficiency testing:

- after packing
- at regular intervals during the working life of the column
- when separation performance deteriorates

Column efficiency testing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent.

For more information about column efficiency testing, refer to the application note *Column efficiency testing (CY13149)*.

Note: *The calculated number of plates will vary according to the test conditions and must only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.*

Sample volume and flow velocity

For optimal results, the sample volume must be at maximum 2.5% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

Method for measuring HETP and A_s

Calculate HETP and A_s from the UV curve (or conductivity curve) as follows:

$$\text{HETP} = \frac{L}{N}$$

L = bed height (cm)

N = number of theoretical plates

$$N = 5.54 \times \left(\frac{V_R}{W_h} \right)^2$$

V_R = retention volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are expressed in the same units

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height (h) is calculated as follows:

$$h = \frac{\text{HETP}}{d_{50v}}$$

d_{50v} = median particle size of the cumulative volume distribution (cm)

As a guideline, a value of < 3 for (h) is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible (A typical acceptable range could be $0.7 < A_s < 1.3$).

A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

Fig. 12, on page 43 shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

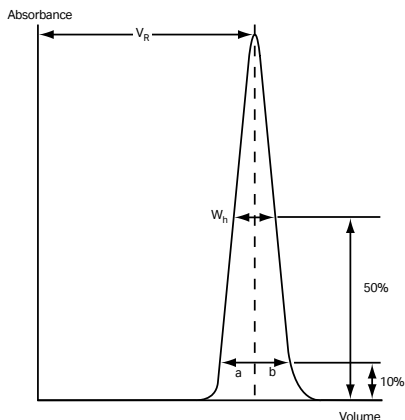


Fig 12. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

6 Maintenance

For best performance of Capto adhere over a long working life time, follow the procedures described below.

Equilibration

After packing, and before a chromatographic run, equilibrate with loading buffer by washing with at least 5 bed volumes, or until the column effluent shows stable conductivity and pH values.

Regeneration

After each step, elute any reversibly bound material with low pH (e.g., 3 column volumes of 0.5 M acetic acid). Regenerate the resin by washing with reversed flow direction until the column effluent shows stable conductivity and pH values.

CIP

CIP is a procedure that removes impurities or contaminants such as lipids, endotoxins, nucleic acids, and precipitated or denatured proteins that remain in the packed column after regeneration. Regular CIP prevents the buildup of impurities or contaminants in the resin bed and helps to maintain the capacity, flow properties, and general performance of Capto adhere. A specific CIP protocol must be designed for each process according to the type of impurities or contaminants present.

It is recommended to perform a CIP:

- before first-time use – especially after packing the column – or after long-term storage
- between cycles

- when an increase in back pressure or a reduction in column performance is observed
- to prevent potential cross-contamination or carry-over, when the same column is used for purification of different proteins or protein lots and batches.

Recommended protocols for removing specific contaminants or impurities are described below.

CIP protocols	
Precipitated hydrophobically bound proteins or lipoproteins	Wash with at least 3 column volumes of 1.0 M NaOH at a maximum flow rate of 150 cm/h with reversed flow direction. Contact time 15 to 30 minutes.
Ionically bound proteins	Wash with 0.5 to 2 column volumes of 2 M NaCl with reversed flow direction.
Lipids and very hydrophobic proteins	Wash with 1-propanol 1% to 5% or isopropanol 5% to 30% with reversed flow direction. 1-propanol has a higher flash point and might be preferred in an industrial environment.
Nucleic acids	Wash with 0.1 M acetate, pH 3, for 2 to 5 column volumes followed by equilibration buffer at neutral pH for 1 to 2 column volumes and wash with 1.0 M NaOH at a maximum flow rate of 150 cm/h with reversed flow direction. Contact time 15 to 30 minutes.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1.0 M NaOH with a contact time of 1 hour is recommended. The CIP protocol for precipitated, hydrophobic bound proteins or lipoproteins removes bound impurities and sanitizes the resin effectively. For more information about the use of NaOH for sanitization, refer to *Application note (CY13951)*.

Storage

Store used resin in its container at 4°C to 30°C. Make sure that the screw top is tightened fully. Packed columns must be equilibrated in 20% ethanol to prevent microbial growth. After storage, equilibrate with at least 5 bed volumes of loading buffer before use.

7 Ordering information

Product	Quantity	Product code
Capto adhere	25 mL	17544410
	100 mL	17544401
	1 L	17544403
	5 L	17544404
	10 L	17544405
	60 L ¹	17544460

¹ Pack sizes available upon request

All bulk resin products are supplied in suspension in 20% ethanol. For additional information, including data file, contact your local Cytiva representative.

Related product	Quantity	Product code
PreDictorCapto adhere, 6 μ L	4 $\times$ 96-well filter plates	28925817
PreDictor Capto adhere, 20 μ L	4 $\times$ 96-well filter plates	28925818
PreDictor Capto adhere, 50 μ L	4 $\times$ 96-well filter plates	28925819
PreDictor Capto adhere Isotherm (2, 4, 6, 8, 20, and 50 μ L, different in different wells within the same plate)		28943282
PreDictor RoboColumn Capto adhere, 200 μ L	One row of eight columns	28986085
PreDictor RoboColumn Capto adhere, 600 μ L	One row of eight columns	28986179
HiTrap Capto adhere	1 $\times$ 1 mL	29400456
HiTrap Capto adhere	5 $\times$ 1 mL	28405844
HiTrap Capto adhere	5 $\times$ 5 mL	28405846
HiScreen Capto adhere	1 $\times$ 4.7 mL	28926981
ReadyToProcess™ Capto adhere (80/200)	1 L	28951109
ReadyToProcess Capto adhere (178/200)	5 L	29146144
ReadyToProcess Capto adhere (251/200)	10 L	28901714
ReadyToProcess Capto adhere (359/200)	20 L	28901716
ReadyToProcess Capto adhere (450/200)	32 L	29212477

Related product	Quantity	Product code
HiTrap MabSelect SuRe	5 × 1 mL	11003493
HiTrap MabSelect SuRe	5 × 5 mL	11003495
HiPrep 26/10 Desalting	1 × 53 mL	17508701
HiPrep 26/10 Desalting	4 × 53 mL	17508702
Tricorn 5/100 column	1	28406410
Tricorn 10/100 column	1	28406415
HiScale 10/40	1	29360550
HiScale 16/20	1	28964441
HiScale 16/40	1	28964424
HiScale 26/20	1	28964514
HiScale 26/40	1	28964513
HiScale 50/20	1	28964445
HiScale 50/40	1	28964444

Accessories	Quantity	Product code
Tricorn Glass Tube 5/100	1	18115306
Tricorn Packing Connector 5-5	1	18115321
Tricorn Packing Equipment 10/100	1	18115325
Packing tube 20 (HiScale 10)	1	29360551
Packing tube 20 (HiScale 16)	1	28986816
Packing tube 40 (HiScale 16)	1	28986815
Packing tube 20 (HiScale 26)	1	28980383
Packing tube 40 (HiScale 26)	1	28964505
Packing tube 20 (HiScale 50)	1	28980251
Packing tube 40 (HiScale 50)	1	28964506

Related literature		Reference
Handbook	Multimodal Chromatography Handbook	CY14738
Data files	Capto adhere	CY11848
	HiScreen prepacked columns	CY13473
	PreDicator 96-well filter plates and Assist Software	CY13663
	HiScale columns	CY13664
Application notes	Optimization of loading conditions on Capto adhere using design of experiments	CY13251
	Efficient purification of the pertussis antigens toxin, filamentous haemagglutinin, and pertactin in chromatography workflows	CY13819
	Two step purification of monoclonal IgG1 from CHO cell supernatant	CY13148
	Selective removal of aggregates with Capto adhere	CY13229
	High-throughput screening and optimization of a multimodal polishing step in a monoclonal antibody purification process	CY13312

Related literature	Reference
Purification of a monoclonal antibody using ReadyToProcess columns	CY20575
Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems	CY13951
Instructions	Packing Capto adhere and Capto MMC using verified methods
	CY29524
	HiScale columns (10, 16, 26, 50) and accessories
	28967470

8 Further information

For the latest news, more product information and our handbooks, visit: [cytiva.com/protein-purification](https://www.cytiva.com/protein-purification)

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